/THE ROLE OF SOLUBLE CARBOHYDRATES
IN LACTIC ACID PRODUCTION/

by

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I am dedicating my entire master's thesis to my boyfriend, Tarek.

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LITERATURE REVIEW

1. Introduction

Ruminal acidosis is a metabolic disease caused by the excessive ingestion of feeds which contain readily fermentable carbohydrates. This occurs most frequently in feeder cattle and high producing dairy herds. Some common names for such a condition are: lactic acidosis, overeating, acute impaction, grain engorgement, founder, and overloading. Following the ingestion of feeds which contain readily available carbohydrates, outward signs of acidosis are anorexia, diarrhea, mucous in the feces, dehydration, incoordination, and even death (Elam, 1976). When acidosis develops in the animal the following physiological changes have been observed:

1. Increased level of lactic acid in the rumen and blood.

2. Increased osmotic pressure in the rumen.

3. Reduction in rumen and blood pH.

4. Destruction of gram negative bacteria and proliferation of gram positive bacteria in the rumen.

5. Reduction in rumen protozoal count.

6. Rumenitis and sloughing of rumen epithelium.

7. Rumen stasis.

8. Reduction in urine pH.

9. Dehydration and hemoconcentration.

(Elam, 1976; Howard, 1981; Dirkson, 1970; Huber, 1976; Slyter, 1978).

A common reason for the occurrence of lactic acidosis is usually attributed to an error in management. The modern feedlot operator or dairy herdsman is confronted with a problem of trying to maximize gain or milk production by feeding a high concentrate diet. This increases the risk of

triggering "lactic acidosis". Management conditions in which acidosis may occur are 1) starting cattle on feed, 2) transitions to a higher concentrate diet, 3) sudden changes in the weather, 4) long periods on a finishing diet, 5) irregular feeding schedules (Dunlop, 1972; Elam, 1976).

2. Chemistry of Lactic Acid

The three-dimensional and non super-imposable forms of lactic acid are indicated below. Both isomers have common chemical properties but differ in some physical aspects (Dunlop, 1972).

COOH

HO-C-H

CH3

L(+)lactic acid

COOH

H-C-OH

CH3
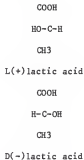
D(-)lactic acid

The dissociation constant is of great importance to the biological environment. Lockwood (1965) reported that lactic acid has a dissociation constant of (1.74×10^{-3}). This value is twice as great as was earlier reported in the Handbook of Chemistry and Physics (8.4×10^{-4}).

Lactic acid in the rumen of sheep and cattle overfed grain was found to occur as both D(-) and L(+) (Dunlop, 1972). Elevated blood lactate concentrations obtained by Dunlop (1972) upon inducement of lactic acidosis consisted mainly of the D isomer.

3. Etiology

(A). CARBOHYDRATE PRECURSORS OF LACTIC ACID - It is anticipated that
the degree of hazard for a given feed would be dependent upon its content of
precursors for lactic acid. Among the carbohydrates which can lead to
accumulation of lactic acid are starch, maltose, sucrose, lactose, cellob-
iose, fructose, and glucose (Dunlop, 1972). Prins and Lankhorst (1976)
reported that formation of lactic acid during fermentation is correlated
with the rate of fermentation and the soluble sugars sucrose, fructose,
glucose, and raffinose which support the highest rates of lactic acid
accumulation.

Krogh induced acidosis by feeding sucrose, lactose, and starch in three
different experiments by studying the microbial changes during acidosis
(Krogh, 1959; Krogh, 1960; Krogh, 1961). Hungate, et al. (1952) isolated
Streptococcus bovis from the rumen when the animals were fed large amounts
of grain or glucose. The organism fermented glucose and produced lactic
acid. Some species of rumen microbes may form lactic acid as one of their
fermentation products, while others are capable of fermenting at least one
of the isomers to volatile fatty acids (VFA) and other end-products.

When readily fermentable carbohydrates are present in the rumen, there
is a rapid fermentation of the carbohydrates to VFA and gaseous products by
the microorganisms. A rapid rise in VFA concentration usually preceeds the
accumulation of lactic acid and a decrease in rumen pH. When the pH falls
below 5.5, many of the normal rumen bacteria (mostly gram negative) and
protozoa are inactivated or die and there is a rapid increase in the number
of streptococci (Counette and Prins, 1979).

Lactic acid is formed primarily through the Embden-Myerhoff pathway in
the rumen. It is known that the LDH-enzymes (lactate dehydrogenase) of the

microorganisms may be either NAD-dependent (Douelle, 1971; Gasser et al., 1970) or NAD-independent (Snoswell, 1963; Doelle, 1971). The LDH enzyme is stereospecific. An organism may produce one isomer or both isomers (Stetter and Kandler, 1973; Stockland and San Clemenente, 1969). When the rate of glycolysis is high, increased intracellular levels of fructose 1,6 diphosphate (FDP) may activate the enzyme LDH (Counette and Prins, 1979). FDP acts by reducing the apparent Km for both pyruvate and NADH (Counette and Prins, 1979). This would shift the reaction toward increased production of lactate.

(B). FEEDS RICH IN READILY FERMENTABLE CARBOHYDRATES — The primary cause of acidosis appears to be the consumption of feeds which are rich in readily fermentable carbohydrates that serve as precursors for the lactic acid intermediate. Grains, fruits, and by-products of these substances are the most common sugar-containing feeds which are used in diets in the United States. While high grain diets are predisposing to acidosis, some grains are worse than others. Wheat is generally considered the worst grain as far as acidosis is concerned. Milo and corn diets are also highly conducive to the occurrence of acidosis, while barley was observed to be the least predisposing (Elam, 1976).

Early research consisted of studies in which animals were engorged with various types of feeds. General observations of the symptoms of "grain engorgement" and rumen lactic acid concentrations were reported earlier (Scarisbrick, 1954; Ryan, 1964; Phillipson, 1942; Phillipson and McAnally, 1942; Allison et al., 1964a). Doses of feed reported to be lethal were 50-60 grams of crushed wheat per kilogram of body weight in undernourished sheep to 75-80 grams per kilogram in well-nourished sheep (Turner and Hoggetts, 1949-1959). Of the six cattle dosed with ground grain or corn at 25-62 grams per kilogram, all developed severe acidosis and one died

(Dunlop, 1970). Experiments with sheep indicated that larger animals were more vulnerable than smaller ones when the dosage was based on body weight (Dunlop, 1970).

A gradual transition from a low concentrate diet to a high concentrate diet is recommended (Allison, 1964). Allison reported that the rate of adaptation to a new diet (high wheat concentrate diet) may be accelerated by intraruminal innoculation with ruminal material from an animal adapted to that diet. Counette and Prins (1979) recommended at least a 14 day adaptation period for an aoimal being switched from a low coocentrate diet to a high concentrate diet. Waroer (1962) found that all major changes io microbial population occurred within 10 days after the ratioo was changed. Annisoo et al. (1959b) estimated that the microbial population adapted to the diet within 3-6 days.

(C). ACCUMULATION OF LACTIC ACID IN THE G.I. TRACT - The biochemical environment of the G.I. lactic acid fermentatioo is not yet fully understood. Previous experiments studyiog lactic acidosis have reported clinical signs rather than the biochemical aspects. When sheep are voluntarily overfed, the proportion of D-lactate normally rises from 20% at pH 6 up to 50% below pH 5 with D-L-lactate concentrations of 100-150 mM (Giesecke et al., 1976). Maioly the D-isomer is produced in the fermentation of most pentoses and hexoses (Prins et al., 1976). Depending on the type of diet and feediog level a considerable portion may escape ruminal cooversion to VFA (Brüggemann and Giesecke, 1968). Ruminants fed high starch diets may have a considerable amount of fermentable carbohydrate escape rumen degradation and will provide substrates for intestinal bacteria (Tubbs, 1965).

(D). TRANSPORT AND ABSORPTION - Absorption of fermentation acids from the gut is faster for the undissociated forms. Intestinal absorption of lactate is via diffusion out of the lumen of the gut (Giesecke and Stangassinger, 1980). Lactate and lactic acid can enter the body via the rumen or the G.I. tract. If the lactic acid enters in the undissociated form, it ionizes at the pH of the plasma. The hydrogen ion will combine with a bicarbonate ion to form carbonic acid which will disociate to CO_2 and H_2O. Thus, absorption of lactic acid reduces the body's reserves of bicarbonate leading to acidosis (Dunlop and Hammond, 1965). However, if sodium lactate is absorbed, alkalosis tends to occur because the lactate anion is metabolized to yield a bicarbonate ion (Dunlop et al., 1969).

(E). TRANSFORMATION OF LACTATE - The conversion of lactic acid into pyruvate is through the NAD-dependent L-lactate dehydrogenase. While, the mitochondrial FAD-containing enzyme, D-2-hydroxy acid dehydrogenase is responsible for the oxidation of D-lactate. D-lactate must pass through the mitochodrial membrane for oxidation (Giesecke and Stangassinger, 1980). This translocation as well as the possible inhibition of D-2-hydroxy acid dehydrogenase by pyruvate and oxaloacetate make the oxidation of D-lactic acid difficult (Cammack, 1970). D-lactate removal is dependent upon the concentrations of pyruvate, enzymes, and various coenzymes (Cammack, 1970).

D-2-hydroxy acid dehydrogenase activity is highest in the ruminant liver and kidney tissues (Cammack, 1969). Rumen epithelial tissue has been shown to oxidize very little D(-)lactic acid (Hinkson et al., 1967; Preston and Noller, 1973). Higher oxidation rates of D(-)lactic acid in rumen epithelium were observed in the absence of L(+)lactic acid (Prins and Lankhorst, 1976). On a total organ weight basis, the liver has the highest activity for D(-)lactic acid while on a dry weight basis, the kidney

exhibits the highest activity. Gluconeogenesis from L(+)lactic acid is twice that of D(-)lactic acid in both tissues (Giesecke and Stangassinger, 1980).

4. Prevention

It is likely that animal as well as microbial factors are responsible for regulating tolerance or adaptation to a new ration (Allison et al., 1964). The common management practice in changing a ruminant's diet from forage to a high or all-concentrate diet is either to limit the amount of concentrates the animal is allowed to consume, or to increase the amount of concentrate in the diet in a stepwise manner until the changeover is complete (Slyter, 1976; Dirkson, 1970; Mullen, 1976). Rumen fluid from adapted animals contains lactic acid utilizing bacteria in sufficient numbers, or are capable of multiplying to sufficient numbers, to prevent accumulation of lactic acid in the rumens of unadapted animals fed a high-energy ration (Huber, 1976). The ruminal microflora and fauna apparently become adapted to this high carbohydrate diet and metabolize glucose as rapidly as it is formed without production of excess lactic acid (Ryan, 1964). The risk of lactic acidosis by concentrate mixtures cannot be predicted from in vitro incubations with single concentrate ingredients (Malestein et al., 1981). Furthermore, it has been demonstrated that in studies on carbohydrate fermentation in the rumen it is necessary to define the sources of starch or sugar used (Malestein et al., 1981).

INTRODUCTION

Ruminal acidosis is a metabolic disease caused by the excessive ingestion of feeds which contain readily fermentable carbohydrates. This condition occurs most frequently in feeder cattle and high producing dairy herds.

It is anticipated that the degree of hazard for a given feed would be dependent upon its content of precursors for lactic acid. Among the carbohydrates that can lead to accumulation of lactic acid are starch, maltose, sucrose, lactose, cellobiose, fructose, and glucose (Dunlop, 1972). Prins and Lankhorst (1976) reported that formation of lactic acid during fermentation was correlated with the fermentation rate and the soluble sugars sucrose, glucose, and raffinose produced the highest lactic acid concentration.

In the United States, grains, fruits, and their by-products are the most common sugar-containing feeds which are used in diets. While high grain diets are predisposing to acidosis, some grains are worse than others. Wheat is generally considered the worst grain as far as acidosis is concerned. Milo and corn diets are also highly con-ducive to the occurrence of acidosis, while barley has been observed to be the least predisposing (Elam, 1976). The risk of lactic acidosis by concentrates mixtures cannot be predicted from in vitro incubations with single ingredients (Malestein et al., 1981). Furthermore, it has been demonstrated in studies on carbohydrate fermentation in the rumen that it is necessary to define the sources of starch or sugar used (Malestein et al., 1982).

Therefore, the purpose of this study was to 1) determine the lactic acid (D and L) production from various carbohydrates, grain and by-products in vitro, 2) to better understand the relationships between soluble carbo-hydrates and lactic acid formation.

SUMMARY

The purpose of this study was to determine the lactic acid (D and L) production from various grain and by-products in vitro, to better understand the relationship between soluble carbohydrates and lactic acid formation. In vitro incubations revealed that hexose sugars exhibited a greater lactic acid production at 12h than pentose sugars. The pentose sugars were not readily fermented to lactic acid. In vitro incubations of grains and by-products revealed a ranking of total lactic acid production at 12h: steam-flaked barley = whole barley = wheat > citrus pulp > beet pulp = corn > high-moisture corn > milo. A segment of this experiment involved the extraction of the soluble fraction via hot (70C) water. The extracted water-soluble fraction and the insoluble fraction were lyphilized and used for in vitro incubations as well as carbohydrate analysis via HPLC. Individual in vitro analysis of the soluble fraction and insoluble fraction revealed that lactic acid concentrations of the insoluble fractions were lower than the soluble fractions (P<.05). Analysis revealed that soluble sugars accounted for only about one-third of the soluble fraction. In vitro incubations were performed on combinations of the least lactic acid provocative insoluble grains (milo, corn, high-moisture corn) with the most lactic acid provocative soluble fractions (steam-flaked barley, whole barley, wheat). The greatest response was seen with the corn insoluble extract. In general, the milo extract and high-moisture corn showed little if any responses to the soluble fraction additions at 12h.

Results indicate that certain grains contain a higher percent of water soluble materials than others. In order to predict lactic acid production, it is important to know the percent water soluble material of the whole grain as well as the fermentable carbohydrates which composite the soluble

fractions. Water soluble materials are important for the initiation of the lactic acid production; thereafter, the production is dependent on the fermentability of the grain starch.

MATERIALS AND METHODS

IN VITRO INCUBATIONS - Steers fitted with rumen cannulae served as the donors of the rumen fluid. Their ration consisted of 80:20 roughage (alfalfa) to concentrate (corn 49.325%, milo 49.325%, dicalcium phosphate 1%, NaCl .25%, vitamin A&D .1%). Upon collection, rumen fluid was strained through four layers of cheesecloth and taken to the laboratory in a pre-warmed thermos flask. Variable quantities of the sugar or feedstuff which had been mixed with McDougal's buffer (pH 6.8) with added urea (.80 g/liter) were inoculated with equal portions of rumen fluid (1:1 mixture) (McDougal, 1949). Samples were contained in plastic centrifuge tubes, gased with CO_2, covered with Bunsen stoppers and incubated in a water bath at 39C. The pH was measured at 4, 8, and 12h and 2ml samples were taken, combined with 4ml of 8% $HClO_4$, and samples were then centrifuged at 10,000xg for 15 min. Samples were stored and frozen at -20 C until analyzed for D(-) and L(+)lactic acid enzymatically (Bergmeyer, 1974). All in vitro incubations were triplicated.

EXPERIMENT 1 IN VITRO LACTIC ACID DETERMINATION OF INDIVIDUAL SUGARS

The effect of sugars on pH and lactic acid production was studied. In this experiment 10 mmoles of each sugar in 30 ml of a 1:1 mixture of fluid were incubated for 12h. Samples of the incubation medium and pH were taken at 4, 8, and 12h for arabinose, ribose, and xylose (1.50g), cellobiose and sucrose (1.71g), stachyose (1.67g), glucose, galactose, fructose, maltose (1.80g), and raffinose (1.98g).

EXPERIMENT 2   IN VITRO D(-) AND L(+)LACTIC ACID PRODUCTION OF GRAINS
AND THEIR BY-PRODUCTS

The effect of a number of feedstuffs and various by-products on pH and
lactic acid production were studied during a 12h incubation. Feedstuffs in
this experiment were ingredients commonly used in commercial concentrate
mixtures. Feed samples were ground through a 1mm screen. Three grams of
each substrate in 30 ml of the 1:1 mixture were used in this portion of the
experiment (citrus pulp, whole barley, steam-flaked barley, wheat, sorghum
grain, corn, beet pulp, and high-moisture corn). Sampling and experimental
procedure were as before.

C) VFA ANALYSIS - VFA analysis was performed on fluid taken at 12h
from the incubation of the whole grain samples from experiment 2. The 8%
$HClO_4$ was precipitated with 6N KOH and centrifuged at 10,000xg for 15min.
The samples were then acidified with 25% metaphosphoric acid (Erwin et al.,
1961), pipetted into a vial and capped. Samples were analyzed by a Hewlett-
Packard gas chromatograph Model 5890A (Hewlett-Packard, Avondale, PA 19311)
using a 10% SP-1200/1% phosphoric acid on a 80/100 Chromosorb (2mm x 6mm)
glass with a 4mm internal diameter column (Supelco, Inc., Bellefonte, PA
16823). Inlet and detector were maintained at 200 C; the oven at 140 C with
a carrier gas (N_2) flow of 40ml/min. Chromatographs were quantified using
a Hewlett-Packard Model 3392A integrator (Hewlett-Packard, Avondale, PA
19311).

EXPERIMENT 3   IN VITRO D(-) AND L(+)LACTIC ACID PRODUCTION OF THE
WATER SOLUBLE CARBOHYDRATE FRACTION (WSCHO) OF EACH GRAIN AND THE GRAIN
MINUS THE WSCHO FRACTION

A) EXTRACTION OF THE WSCHO FRACTION - Twenty-five grams of grain were
placed in a plastic centrifuge bottle and hot water (60-70 C) was added to
the grain. The bottle was shaken for 3-5 min. The samples were then

centrifuged at 3000xg. The supernatant was poured off. Hot water was again added to the bottle for additional extraction and the entire procedure was repeated. The supernatant fluid and extracted grain were frozen. The samples were then lyophilized and stored until analysis. Preliminary experiments indicated 2 extractions yielded adequate quantitative recovery of water soluble materials.

B) IN VITRO INCUBATIONS - Comparisons of the D(-) and L(+)lactic acid production of the extracted grain (.67g) and their WSCHO fractions (.67g) were compared with in vitro incubations. Substrates were individually incubated in a 20ml (1:1 mixture) of McDougal's buffer plus rumen fluid for 12h. Sampling and experimental design were as described for experiment 1. VFA analysis was performed on all 12h extracted grain samples as described in experiment 2.

EXPERIMENT 4 IN VITRO LACTIC ACID DETERMINATION OF THE MOST LACTIC ACID PROVOCATIVE WSCHO FRACTIONS COMBINED WITH THE LEAST LACTIC ACID PROVOCATIVE EXTRACTED GRAINS

The determination of the combinations of this experiment were decided from the experimental results obtained from experiments 2 and 3. One-tenth of a gram of the most lactic acid provocative WSCHO fractions was combined with .9g of the least lactic acid provocative extracted grains. At the same time, 1g incubations of the extracted grains and whole grains of the least lactic acid provocative substrates were individually incubated and compared to the WSCHO fractions plus the extracted grains. The substrates were incubated in 20ml of the 1:1 mixture for 12h. Sampling and experimental design were as described for experiment 1.

EXPERIMENT 5   DETERMINATION OF THE WSCHO FRACTION OF THE INDIVIDUAL
GRAINS VIA HIGH PRESSURE LIQUID CHROMATOGRAPHY

Five tenths of a gram of lyophilized WSCHO fractions was dissolved in
10ml of deionized water. All samples except the steam-flaked barley WSCHO
fraction were filtered through a .20 micron Gelman disc (Gelman Sciences,
Inc., Medical Sciences Division, Ann Arbor, MI 48106) and run through
a Sep-Pak (Waters Inc., Millipore Products Division, Bedford, MA. 01730).
The Sep-Pak was prepared by slowly running 2ml of 100% acetonitrile followed
by 10ml of the previously filtered eluent which was then discarded. Two
milliliters of the previously filtered eluent were again run through the
Sep-Pak and saved for analysis. Because the steam-flaked barley WSCHO
fraction was an extremely viscous slurry, a different sample preparation was
used. The slurry was poured into a Centricon centrifugal microcontainer
with a 10,000 molecular weight cutoff (Amicon Corporation, Scientific System
Division, Danvers, MA 01923) and centrifuged at 7,000xg for 2-4 hours at
5C.

Samples were injected into a Beckman 100A injector system with a 20
microliter loop. The detector used was a Beckman refractive index detector
- Model 153 (Beckman Instruments, Inc., Altex Scientific Operations,
Berkeley, CA 94710). The carbohydrates were separated by a Brownlee Amino
Spheri-5 carbohydrate column 25cm by 4mm with a 3cm precolumn (Brownlee
Labs, Rainin Instrument Company, Inc., Woburn, MA 01801). The solvent
was 80% acetonitrile and 20% water. It was pumped through a Beckman 100A
pump with a flow rate of 1 ml/minute (Beckman Instruments, Inc.). Chromato-
grams were quantified using a Hewlett-Packard 3390A integrator (Hewlett-
Packard, Avondale, PA 19311).

STATISTICAL ANALYSIS

Data were analyzed using analysis of variance (SAS Institute, Inc., Cary, NC 27511). Variation was partitioned into repetition and substrate effects at 4, 8, and 12h. Least square means and differences between the means were obtained for all samples. Linear contrasts were used for data analysis in experiments 3 and 4.

RESULTS

EXPERIMENT 1

The pH values for all sugars declined relative to control (Figure 1)
progressively at 4, 8, and 12h. Cellobiose, galactose and maltose exhibited
the highest L(+)lactic acid concentrations at 12h. L(+)lactic acid concen-
trations generally increased and were greater at 12h for (P<.001) for
maltose, cellobiose, glucose, fructose, raffinose, stachyose, sucrose
and galactose compared with arabinose, xylose, and ribose (Figure 2).
D(-)lactic acid concentrations generally followed increases in L(+)lactic
acid with fructose, maltose, and sucrose exhibiting the highest concentra-
tions (Figure 3). D(-) concentrations were greater at 12h (P>.0001) for
cellobiose, fructose, galactose, glucose, maltose, raffinose, stachyose, and
sucrose compared with ribose, xylose, and arabinose. The concentration of
D(-)lactic acid for fructose was greater (P<.05) than sucrose. The pentose
sugars ribose, xylose, and arabinose were not readily fermented to lactic
acid. The possible explanation would exist in the metabolism of these
sugars via the pentose-phosphate pathway yielding volatile fatty acids
instead as evidenced by a decline in pH (Lehninger, 1975). Concentrations
of D(-)lactic acid were generally 20-30% of the L(+)lactic acid concentra-
tions.

EXPERIMENT 2

The pH values for the individual grains decreased at 4, 8, and 12h
(Figure 4). After 12h, tubes containing steam-flaked barley, whole barley,
and wheat fermentations contained more L(+)lactic acid (P<.05) than those
with beet pulp, corn, high-moisture corn, or sorghum grain (Figure 5).
After 12h, wheat, whole barley, and steam-flaked barley exhibited the
greatest D(-)lactic acid concentrations and were (P<.001) greater than

high-moisture corn, milo, corn, and beet pulp (Figure 6). Corn, milo, and high-moisture corn had similar L(+)lactic acid concentrations (P<.05) at 4, 8, and 12h. The 12h rankings for the total D(-) and L(+)lactic acid would be: steam-flaked barley = whole barley = wheat > citrus pulp > beet pulp = corn > high-moisture corn > milo. At 12h, whole barley, steam-flaked barley, and milo grain contained the highest acetate and propionate concentrations, respectively. These three grains were greater (P<.10) than wheat, corn, or the blank in acetate concentrations and greater (P<.1) than corn in propionate concentrations. At 12h, steam-flaked barley, high-moisture corn, and milo grain exhibited the greatest butyrate concentrations and were higher than coro (P<.1), or whole barley (P<.05). The greatest total VFA concentrations were seen with whole barley, steam-flaked barley, and milo grain and were not higher than any of the other grains (P<.1) (Figure 7).

EXPERIMENT 3

All grain extracts exhibited a decrease in pH at 4, 8, and 12h (Figure 8). Most grain WSCHO fractions decreased in pH after 12h; however, the high-moisture corn, sorghum grain, and wheat did not exhibit a decline at 12h. Concentrations of L(+) and D(-)lactic acid concentrations at 4, 8, and 12h for extracted grains were lower (P<.05) than the WSCHO fraction (Figures 9 and 10). Whole barley and wheat extracted grains exhibited the highest L(+)lactic acid concentrations at 12h and were higher than milo, high-moisture coro, aod coro extracted grains (P<.05). The WSCHO fractioos of corn, whole barley, and steam-flaked barley exhibited the highest L(+)lactic acid at 12h, but were not statistically different (P<.05) from the other WSCHO fractions. Likewise, whole barley and wheat exhibited the highest D(-)lactic acid concentrations at 12h, but were not statistically different than any of the extracted grains. Steam-flaked barley, whole

barley, and corn WSCHO fraction exhibited the highest D(-)lactic acid concentratons at 12h, and were higher than the high-moisture corn and milo WSCHO fractions (P<.05).

Milo, corn, and high-moisture corn extracted grains produced the greatest concentrations of acetate, propionate, butyrate, and total VFA concentrations, but were not statistically different than the other grains (P<.05) at 12h (Figure 7).

EXPERIMENT 4

Whole barley, steam-flaked barley, and wheat WSCHO fractions combined with the corn extract showed the greatest response in decreased pH values, and increased L(+) and D(-)lactic acid concentrations at 12h (P<.05). In general, the milo extract showed little if any response to the WSCHO fraction additions at 12h (Figures 11, 12, 13).

EXPERIMENT 5

The composition of the WSCHO fractions for each individual grain are given in Table 1. Of the whole grains, the freeze-dried, water-soluble fraction composited 4.79% corn, 9.12% steam-flaked barley, 9.17% whole barley, 10.28% high-moisture corn, 10.13% wheat, and 4.98% sorghum grain.

The carbohydrate analysis accounted for only one-third of the WSCHO fraction. All sugars were separated except maltose and cellobiose (maltose plus cellobiose). Because of their size and the slow flow rate of the solvent, raffinose and stachyose were not identified.

All grains except high-moisture corn contained fructose, glucose, and maltose plus cellobiose. This is possibly due to the prior ensiling process. Wheat contained the highest amounts of fructose, glucose, and maltose plus cellobiose.

DISCUSSION

The results in experiment 1 indicate that glucose, galactose, and sucrose had the greatest L(+)lactic acid accumulation. The highest rates of D(-)lactic acid accumulation were observed with glucose, fructose, and sucrose. Prins and Lankhorst (1976) observed the greatest L(+)lactic acid accumulation with sucrose, fructose, and glucose, respectively, and the greatest accumulation of D(-)lactic acid with sucrose, glucose, and fructose, respectively.

Prins and Lankhorst (1976) incubated sugars in vitro with rumen fluid for 1h in a .2% w/v media and reported higher proportions of D(-)lactic acid as opposed to L(+)lactic acid. Dennis et al. (1981) reported that soluble sugars such as fructose, galactose, sucrose, lactose, and mannose were fermented readily to lactic acid when incubated 9h in vitro with rumen fluid from a roughage fed animal. Of the hexose sugars, fructose and galactose produced the most lactic acid, which agrees with the results found in this experiment. The relative proportions of L(+) to D(-)lactic acid agree with the findings of Dennis et al. (1976), but do not agree with the predominance of D(-)lactic acid reported by Prins and Lankhorst (1976). In no case were we able to see a greater D(-) than L(+)lactic acid with simple sugars. Prins and Lankhorst used smaller amounts of substrates and incubated with only rumen fluid. In our work, L(+)lactic acid is usually formed first, followed by the onset of D(-)lactic acid formation, as was described by Giesecke and Stangassinger (1980).

Dennis et al. (1981) incubated ground sorghum grain, ground corn, and ground wheat. Ground wheat caused the greatest accumulation of lactic acid. In experiment 2, wheat produced a greater accumulation of lactic acid in comparison to corn or sorghum grain.

CONCLUSIONS

This experiment suggests that the WSCHO fraction plays a role in the initiation of lactic acid production and thereafter is dependent on a fermentable starch. For example in experiment 4, corn exhibited the greatest response when additions of highly fermentable WSCHO fraction were incubated. However, milo and high-moisture corn did not exhibit a response because milo starch apparently is not fermented rapidly and high-moisture corn is perhaps lacking the fermentable starch source. High-moisture corn and milo were both fermented because both yielded large increases in VFA concentrations. However, the breakdown must have been at a rate that would limit a build-up of glucose in the media or lactic acid production would have predominated (Counette, 1979).

The ability to predict whether a grain is lactic acid provocative does not appear to lie solely in the water soluble fraction. Grains such as wheat and barley contain nearly twice as much composited water-soluble fraction as corn; however, in vitro studies would indicate that the corn WSCHO fraction is comparatively lactic acid provocative to wheat and barley. The grain residue of wheat and barley are exceedingly more fermentable than corn residue. Addition of WSCHO from wheat to this corn residue indicated it had the ability to support a high level of lactic acid production once enough soluble materials were present for initiation. Whether differences in corn or grain sorghum variety would yield differences in the amount of WSCHO fraction and affect the feeding value remains to be determined.

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Figure 1. pH of sugars at 4, 8, and 12h (experiment 1).

blank (BL) 7.29+0.18 (means ± standard error at 12h),
arabinose (A) 6.31+0.18, ribose (RI) 6.59+0.18,
xylose (X) 6.40+0.23, fructose (F) 4.45+0.18,
galactose (GA) 4.45+0.18, glucose (GL) 4.38+0.18,
cellobiose (C) 4.44+0.18, maltose (M) 4.47+0.18,
sucrose (SU) 4.41+0.18, raffinose (RA) 4.49+0.18,
stachyose (ST) 4.51+0.18.

27

pH

Figure 2. L(+) lactic acid concentrations at 4, 8 and 12h
(experiment 1).

blank (BL) 0.00±11.58 (means ± standard error at 12h),
arabinose (A) 7.84±11.58, ribose (R) 0.00±11.58,
xylose (X) 18.17±14.40, fructose (F) 93.14±11.58,
galactose (GA) 116.50±11.58, glucose (GL)
114.0±11.58, cellobiose (C) 117.28±11.58,
maltose (M) 121.08±11.58, sucrose (SU)
103.10±11.58, raffinose (RA) 111.55±11.58,
stachyose (ST) 92.25±11.58.
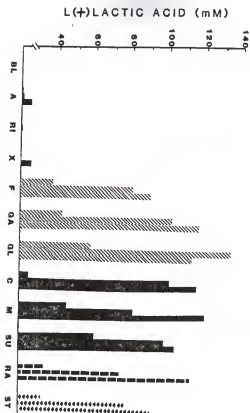
L(+)LACTIC ACID (mM)

Figure 3.   D(-) lactic acid concentrations at 4, 8 and 12h
            (experiment 1).

            blank (BL) 0.00±4.29 (means ± standard error at 12h),
            arabinose (A) 0.86±4.29, ribose (RI) 0.00±4.29,
            xylose (X) -0.683±5.34, fructose (F) 51.93±4.29,
            galactose (GA) 33.52±4.29, glucose (GL) 35.97±4.29,
            cellobiose (C) 35.09±4.29, maltose (M) 42.23±4.29,
            sucrose (SU) 37.37±4.29, raffinose (RA) 33.52±4.29,
            stachyose (ST) 33.34±4.29.

D(-) LACTIC ACID (mM)
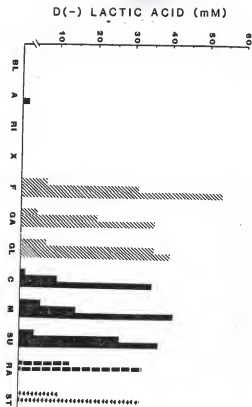
Figure 4. pH of grains and by-products at 4, 8 and 12h
(experiment 2).

blank (BL) 7.40±0.096 (means ± standard error at 12h),
corn (CN) 5.14±0.096, high-moisture corn (HC) 5.70±0.096,
sorghum grain (SG) 5.36±0.096, wheat (WH) 4.93±0.096,
steam-flaked barley (FB) 4.59±0.096, whole barley (WB)
4.59±0.096, beet pulp (BP) 5.52±0.096, citrus pulp (CP)
5.13±0.096.

pH
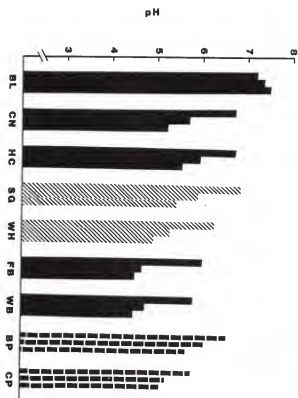
Figure 5. L(+) lactic acid concentrations of grains and by-
products at 4, 8, and 12h (experiment 2).

blank (BL) 0.00+12.57 (means ± standard error at 12h),
corn (CN) 22.20+12.57, high-moisture corn (HC) 0.00+12.57,
sorghum grain (SG) 1.23+12.57, wheat (WH)79.22+12.57,
steam-flaked (FB) 79.22+12.57, whole barley (WB)
91.17+7.35, beet pulp (BP) 39.48+12.57, citrus pulp (CP)
36.86+12.57.
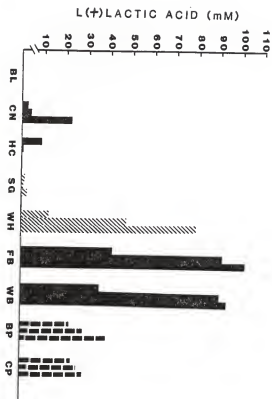
L(+)LACTIC ACID (mM)

Figure 6. D(-) lactic acid concentrations of grains and by-
products at 4, 8 and 12h (experiments 2).

blank (BL) 0.00+2.57 (means ± standard error at 12h),
corn (CN) 8.02+2.57, high-moisture corn (HC) 0.00+2.57,
sorghum grain (SG) 0.00+2.57, wheat (WH) 31.05+2.57,
steam-flaked barley (FB) 24.71+2.57, whole barley
(WB) 30.50+2.57, beet pulp (BP) 2.03+2.57,
citrus pulp (CP) 21.52+2.57.

D(-)LACTIC ACID (mM)
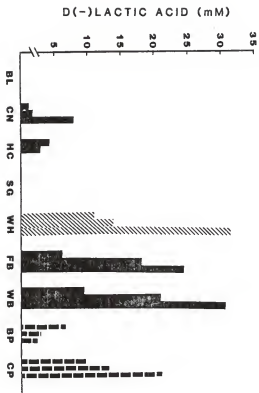
Figure 7.   Total VFA concentration from whole grain samples
(experiment 2) and grain insoluble fractions (extract)
(experiment 3).

whole grain blank (G) 21.07±16.82 (means ± standard error
at 12h), grain extract blank (E) 42.51±13.53, corn (CG)
65.43±13.53, corn extract (CE) 115.45±13.53, high-moisture
corn (HG) 74.88±13.53, high-moisture corn extract (HE)
122.78±13.53, sorghum grain (MG) 77.58±13.53, sorghum
grain extract (ME) 114.38±13.53, steam-flaked barley (SG)
89.47±13.53, steam-flaked extract (SE) 69.69±13.53, whole
barley (BG) 94.52±13.53, whole barley extract (BE)
80.97±13.53, wheat (WG) 44.95±16.82, wheat extract (WE)
99.28±13.53.

TOTAL VFA (mM)

G = WHOLE GRAIN
E = EXTRACTED GRAIN
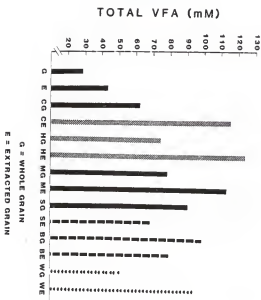
Figure 8. pH of soluble (WSCHO) fractions and grain insoluble
fractions (extracts) at 4, 8, and 12h (experiment 3).

blank (BL) 6.92 ± 0.21 (means ± standard error at 12h),
corn extract (CG) 6.25 ± 0.21, high-moisture corn extract
(HG) 5.80 ± 0.21, sorghum grain extract (MG) 6.13 ± 0.21,
steam-flaked barley extract (SG) 5.78 ± 0.21, whole barley
extract (BG) 5.23 ± 0.21, wheat extract (WG) 5.35 ± 0.21, corn
WSCHO (CW) 5.52 ± 0.21, high-moisture corn WSCHO (HW)
6.59 ± 0.21, sorghum WSCHO (MW) $6.15 \pm$, steam-flaked barley
WSCHO (SW) 5.31 ± 0.21, whole barley WSCHO (BW) 5.01 ± 0.21,
wheat WSCHO (WW) 5.78 ± 0.21.
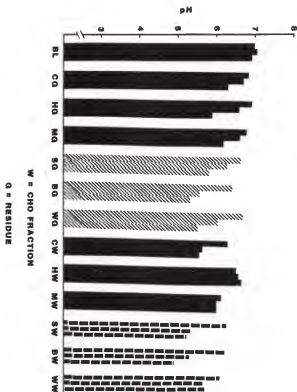
Figure 9.   L(+)lactic acid concentrations of soluble fractions (WSCHO)
and insoluble fractions (extracts) at 4, 8, and 12h
(experiment 3).

blank (BL) 0.00±8.16 (means ± standard error at 12h),
corn extract (CG) .82±8.16, high—moisture corn extract
(HG) 0.00±8.16, sorghum extract (MG) 0.00±8.16, steam—
flaked barley extract (SG) 8.09±8.16, whole barley extract
(BG) 32.59±8.16, wheat extract (WG) 28.46±8.16, corn WSCHO
(CW) 32.78±8.16, high—moisture corn WSCHO (HW) 14.12±8.16,
sorghum WSCHO (MW) 23.66±8.16, steam—flaked barley WSCHO
(SW) 28.89±8.16, whole barley WSCHO (BW) 32.21±8.16, wheat
WSCHO (WW) 23.03±8.16.

43
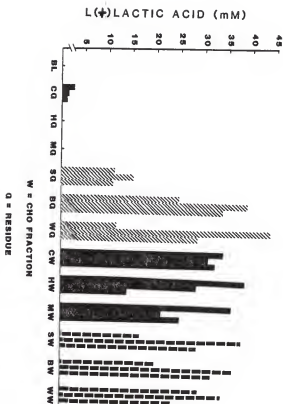
Figure 10.   D(-)lactic acid concentrations of soluble fractions
(WSCHO) and insoluble fractions (extracts) at 4, 8, and
12h (experiment 3).

blank (BL) 0.00 25.87 (means ± standard error at 12h),
corn extract (CG) 2.93±25.87, high-moisture corn extract
(HG) 0.00±25.87, sorghum extract (MG) 0.00±25.87, steam-
flaked barley extract (SG) 2.88±25.87, whole barley extract
(BG) 33.69±25.87, wheat extract (WG) 8.58±25.87, corn WSCHO
(CW) 82.90±25.87, high-moisture corn WSCHO (HW) 24.26±
25.87, sorghum WSCHO (MW) 15.64±25.87, steam-flaked barley
WSCHO (SW) 132.92±25.87, whole barley WSCHO (BW) 95.35±
25.87, wheat WSCHO (WW) 32.87±25.87.

45
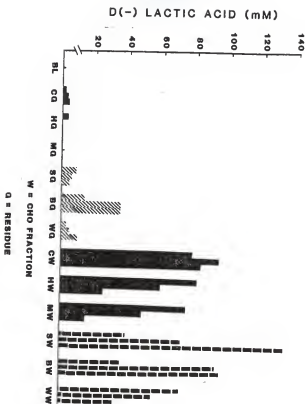
Figure 11. pH values at 4, 8, 12h (experiment 4).

blank (BL) 7.29+0.12 (means ± standard error at 12h),
corn (C) 6.06+0.12, corn extract (CG) 5.34+0.12, corn
extract plus steam-flaked barley WSCHO fraction (CS)
5.10+0.12, corn extract plus whole barley WSCHO fraction
(WB) 5.03+0.12, corn extract plus wheat WSCHO fraction
(CW) 4.98+0.12, high-moisture corn (H) 5.91+0.12,
high-moisture corn extract (HG) 5.67+0.12, high-
moisture corn extract plus steam-flaked barley
WSCHO extract fraction (HS) 5.63+0.12, high-moisture
corn extract plus whole barley WSCHO fraction (HS)
5.47+0.12, high-moisture corn extract plus wheat
WSCHO fraction (HW) 5.51+0.12, sorghum grain (M)
5.57+0.12, sorghum grain extract (MG) 5.67+0.12,
sorghum grain extract plus plus steam-flaked barley
WSCHO fraction (MS) 5.60+0.12, sorghum grain extract
plus whole barley WSCHO fraction (MB) 5.52+0.12,
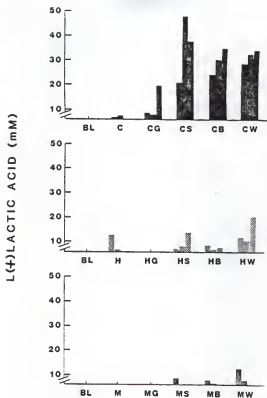sorghum-grain extract plus wheat WSCHO fraction (MW)
5.50+0.12.
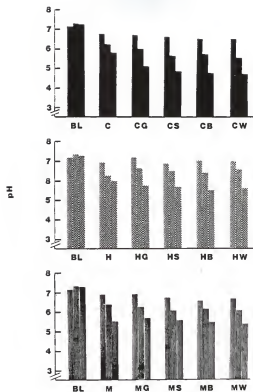
Figure 12. L(+) lactic acid values at 4, 8, and 12h (experiment 4).

blank (BL) 0.00+5.53 (means ± standard error at 12h), corn (C) 0.04+5.53, corn extract (CR) 7.93+5.53, corn extract plus steam-flaked barley WSCHO fraction (CS) 37.01+5.53, corn extract plus whole barley WSCHO fraction (CB) 34.56+5.53, corn extract plus wheat WSCHO fraction (CW) 33.24+5.53, high-moisture corn (H) 0.00+5.53, high-moisture corn extract (HG) 0.00+5.53, high-moisture corn extract plus steam-flaked barley WSCHO fraction (CS) 3.50+5.53, high-moisture corn extract plus whole barley WSCHO fraction (HB) 0.93+5.52, sorghum grain (M) 0.00+5.52, sorghum grain extract (MG) 0.00+5.52, sorghum grain extract plus steam-flaked barley WSCHO fraction (MS) 0.00+5.53, sorghum grain extract plus whole barley WSCHO fraction (MB) 0.00+5.53, sorghum grain extract plus wheat WSCHO fraction (MW) 0.00+5.53.

L(+)LACTIC ACID (mM)

BL C CG CS CB CW

BL H HG HS HB HW

BL M MG MS MB MW

Figure 13. D(-) lactic acid values at 4, 8, and 12h (experiment
          4).

          blank (BL) 0.00±1.76 (means ± standard error at 12h),
          corn (C) 0.00±1.76, corn extract (CG) 1.13±1.76, corn
          extract plus steam-flaked barley WSCHO fraction (CS)
          8.68±1.76, corn extract plus whole barley WSCHO fraction
          (CB) 6.94±1.75, corn extract plus wheat WSCHO fraction
          (CW) 10.06±1.76, high-moisture corn (H) 0.00±1.76,
          high-moisture corn extract (HG) 0.00±1.76, high-
          moisture corn extract plus steam-flaked barley WSCHO
          fraction (CS) 0.12±1.76, high-moisture corn extract
          plus whole barley WSCHO fraction (HB) 1.28±1.76,
          high-moisture corn extract plus wheat WSCHO fraction
          (HW) 0.00±1.76, sorghum grain (M) 0.00±1.76, sor-
          ghum grain extract (MG) 0.00±1.76, sorghum grain
          extract plus steam-flaked barley WSCHO fraction (MS)
          0.00±1.76, sorghum grain extract plus whole barley
          WSCHO fraction (MB) 0.00±1.76, sorghum grain extract
          plus wheat WSCHO fraction (MW) 0.37±1.76.
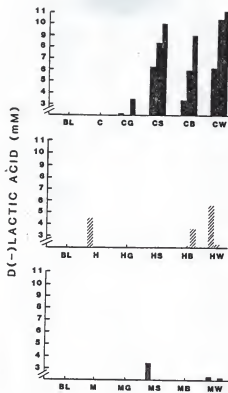
Table 1. Percent soluble materials extracted from the grain and
percent sugars which composite the water-soluble fraction.

			percent of sugars in soluble fraction[3]						
Grain[1]	percent[2]	R	X	A	F	GL	SU	M+C[4]
WHEAT	7.36	.18	.07	.33	.83	1.14	---	.81
MILO	4.98	---	---	---	.31	.59	---	.26
SFB	9.12	.18	.30	.14	.14	.36	.68	.70
WB	9.17	.16	.12	.33	.22	.41	.53	.75
CORN	4.79	---	---	---	.22	.67	.35	.50
HMC	10.28	---	.04	.19	---	.49	---	.21

1 grains: Wheat, sorghum grain (milo), steam-flaked barley
(SFB), whole barley (WB), corn, high-moisture corn
(HMC).

2 Percent of whole grain that was water soluble.

3 Percent of each sugar contained in the soluble fraction as
determined by HPLC analysis.

4 Sugars contained in the soluble fraction ribose (R), xylose (X),
arabinose (A), fructose (F), glucose (GL), sucrose (SU), and
maltose plus cellobiose.

APPENDIX

HEAT STRESS EFFECTS ON

GUT

NUTRIENT ABSORPTION

INTRODUCTION

The detrimental effects of heat stress on livestock productivity have been well documented (Collier (1981), Thompson (1973), Roman-Ponce (1977a)). Studies conducted in environmentally-controlled chambers, have identified upper critical temperatures for many production traits. These critical temperatures normally fall between 24 and 27C (Fuquay, (1981)). Roman-Ponce et al. (1978) studied the effects of thermal stress (shade versus no shade conditions) on dairy cows. The results indicate that the average rectal temperatures and respiration rates per minute were lower in animals given access to shade. It was also found that thermal stress reduces fertility and uterine blood flow after injection of estradiol.

Portal blood flow and net absorption of various dietary nutrients have been studied in various species (Huntington (1980), Webster (1973), McGilliard (1971). These reports have quantified absorption of dietary nutrients in natural environments. However, there is limited information available on nutrient metabolism in heat-stressed ruminants. Sands et al. (1982) determined the contribution of blood glucose to heat production and found there is a decreased turnover rate of blood glucose in sheep exposed to 30C with a relative humidity of 70%.

It was the intent of this paper to focus on the influence of environmental temperature on portal blood flow (PBF) and nutrient absorption, and to better understand the relationships between nutrient absorption and heat stress.

MATERIALS AND METHODS

Three Hereford heifers (350kg) were surgically fitted with rumen fistulae, abdominal aorta, hepatic portal, and mesenteric venous catheters (McGillard, 1972). Animals were fed an 85% concentrate and 15% roughage diet with Rumensin added at 44mg/kg (Table 1).

The experiment was divided into a 2d heat stress period (18-35C) and a 2d control period (18C). Intakes during the control period were restricted to what the animals consumed during the heat stress period.

Sampling period

The schedule for sampling and experimental changes are presented in Figure 1. Animals were maintained in an environmentally-controlled chamber. Animals were aacclimated for 48h at 18C and blood samples were taken twice at 0 and 1h (samples (1) and (2)) beginning the heat stress period (HSP). The temperature was increased to 27C and samples at 4 and 5h (samples (3) and (4)) were taken. Finally, the temperature was raised to 35C and samples at 8, 24, 28, and 32h (samples 5-8) were taken. Following sample (8), the temperature was lowered to 18C for a 24h acclimation period. The temperature was maintained at 18C and samples (9-16) were taken, respectively with no increase in temperature constituting the CP. A 48h acclimation at 18C followed the CP. The sampling times were replicated for both the heat stress period and control period.

Sampling procedure

Blood flow was monitered via para-aminohippuric acid. Para-aminohippuric acid (10% w/v) was titrated to pH 7.4 and filtered through a 45 micron filter prior to use. At the beginning of each infusion, a 15ml priming dose was followed by a continuous infusion into the mesenteric venous catheter (Katz and Bergman, 1969) at a rate of .7ml/min for at least 20-30 min prior to sampling.

Respiration rate, heart rate, rectal temperature, and rumen pH were measured 20-30 min before each blood sampling time. Rumen fluid samples were collected using a suction strainer (Raun and Burroughs, 1962) and the pH was taken directly and recorded. A pair of 35ml blood samples were collected simultaneously from the hepatic portal and abdominal aorta catheters into heparinized syringes. Catheters were then refilled with a heparinized saline solution.

Whole blood samples were deproteinized with one-tenth volume 6N $HCLO_4$, centrifuged for 15 min (15,000xg), neutralized with 6N KOH and recentrifuged. The supernatent was poured off and analyzed immediately for glutamine (Lund, 1974) and glutamate (Bernt and Bergmeyer, 1974). The remainder was stored frozen (-20C) and used for analysis of PAH colormetrically (Harvey and Brothers, 1962). Blood (L+) lactate concentrations were determined enzymatically (Bergmeyer, 1974).

The rumen fluid was deproteinized with 25% metaphosphoric acid (Erwin et al., 1961), centrifuged for 15 min at 15,000xg, and stored until VFA analysis by gas chromatography (Hewlett-Packard, 5890A, Palo Alto, Ca.) using 10% SP-1200/1% H_3PO_4 on an 80/100 Chromosorb (2mx6mm glass with a 4mm internal diameter) column (Supelco Inc., Bellefonte, Pa., 16823). Inlet and detector were maintained at 200C; the oven at 140C with a carrier gas (N_2) flow of 40ml/min.

Statistical analysis and calculations

PBF and net absorption from portal-drained viscera were calculated from equations presented by Katz and Bergman, (1975). Data were partitioned into animal, repetition, and sampling time. Comparisons were made within and between sampling periods using appropriate contrasts.

RESULTS AND DISCUSSION

Intakes during the CP were limited to the amount of feed the animal consumed during the HSP. Intakes averaged $4.3 \pm .8$ and $3.3 \pm .6$ (kg of dry matter$\pm$SE) for the HSP and CP, respectively (Table 1).

McDowell et al. (1969) reported a 5% reduction in concentrate consumption and a 22% decline in hay consumption by lactating Holstein cows increased from 18-30C. In contrast, Sanos et al. (1982) reported no decline in intakes when sheep were exposed to 20C and then 30C.

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when the temperature was increased from 1.6 to 37.7C, respectively, while maintaining a controlled intake throughout the experiment. Kelley stated that one factor that may be observed in animals which may be heat tolerant is the minimization in the variability of ruminal TVFA concentrations.

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Bensadoun and Reid (1962) studied the correlation between VFA concentrations and PBF in sheep. Although rumen VFA concentration curves and PBF patterns showed similiar trends, the correlation coefficients (.20-.41) were not significant. Our findings suggest that the correlation coefficient between PBF and TVFA are also non-significant (.02) in cattle.

Arterial glutamate concentrations (Figure 11) were lower for the HSP samples versus the CP samples except for the HSP sample (1) (P<.05). Differences in arteriovenous (A-V) concentrations (Figure 12) were higher for HSP sample (8) compared to the CP sample (16) (P<.05). No differences between HSP and CP were observed for the net portal absorption (Figure 13) of glutamate (P<.05).

Arterial glutamine (Figure 14) differences were lower for the HSP samples (5-8) versus the comparable samples of the CP (13-16) (P<.05) and HSP sample (2) versus CP sample (10) (P<.05). No differences between the CP and HSP were observed for glutamine A-V differences (Figure 15) or the net portal absorption values for glutamine (Figure 16). Mean net portal absorption values for glutamine were (9.6±3.2) and glutamate were (-4.7±10.4mM). Windmueller et al. (1974) reported that glutamine was the major amino acid removed by the gut. Its major role in the gut metabolism is known to result in the production of citrulline, alanine, proline, and ammonia. Heitmann and Bergman (1980) suggested that hepatic amino acid metabolism is continuous and peripheral tissues may act secondarily to maintain homeostatis. Our results indicate that gut uptake of glutamate and glutamine is continuous.

Arterial L(+) lactate (Figure 17) concentrations were lower at 27C than 18C during the HSP (P<.01). A-V differences of L(+) lactate (Figure 18) concentrations and net portal absorption (Figure 19) values showed no statistical values significances (P>.05). The mean absorption values for L(+) lactate were 72.34 mmoles per hour. Huntington et al. reported absorption values of 31.66 mmoles per hour with steers fed an 85% concentrate diet. In this experiment, the L(+) lactate was absorbed rather than utilized.

CONCLUSIONS

In summary, the results conclude:

Rectal temperature, respiration rates, and pulse rates increased during the HSP ($P<.0001$, $P<.0001$, $P<.05$). Rumen pH values were lower for the HSP (5.78-6.10) as compared to the CP (5.88-6.68) ($P<.01$). TVFA were lower only for the first sample taken at 35C (HSP) ($P<.01$). The acetate concentration was lower only for the first sample (5) at 35C ($P<.01$). Concentrations of the propionate were not lower for the HSP ($P<.05$). Concentrations of butyrate were higher for the 35C (HSP) samples than the CP (18) samples ($P<.01$). PBF differences were observed only for 18C sample (2) of the HSP which was higher than the 18C (10) of the CP ($P<.01$). The correlation between PBF and TVFA was not significant (.02). No differences between HSP and CP were observed for the net portal absorption of glutamate, glutamine or L(+) lactate ($P<.05$). The gut uptake of glutamine and glutamate was continuous. Furthermore, L(+) lactate was absorbed rather than utilized.

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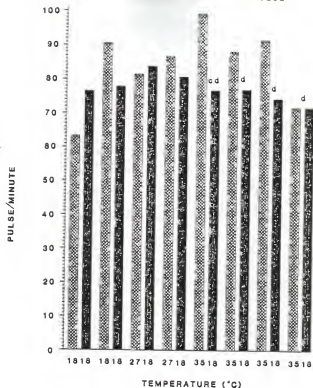
Figure 1. Experimental sampling procedure.

Figure 2. Heat stress effects on rectal temperature.

c. differences between samples at 18C versus 35C (P<.05).

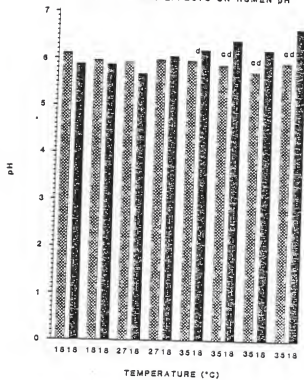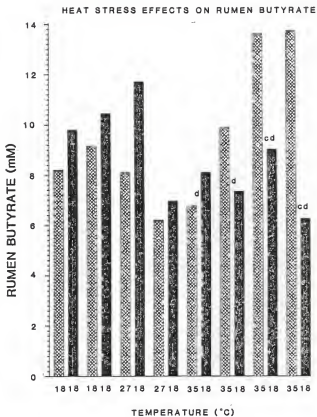d. differences between the HSP and CP (P<.05).

HEAT STRESS EFFECTS ON RECTAL TEMPERATURE

Figure 3. Heat stress effects on respiration.

b. differences between samples at 18C versus 27C (P<.05).

c. differences between samples at 18C versus 35C (P<.05).
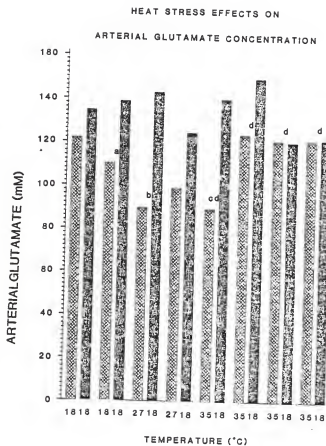
d. differences between the HSP and CP (P<.05).

HEAT STRESS EFFECTS ON RESPIRATION

Figure 4. Heat stress effects on pulse.

c. differences between samples at 18C versus 35C (P<.05).

d. differences between the HSP versus CP (P<.05).

Figure 5. Heat stress effects on rumen pH.

c. differences between samples at 18C versus 35C (P<.05).

d. differences between the HSP and CP (P<.05).

HEAT STRESS EFFECTS ON RUMEN pH

Figure 6. Heat stress effects on total VFA.

c. differences between samples at 18C versus 35C (P<.05).

HEAT STRESS EFFECTS ON TOTAL VFA

RUMEN TVFA (mM)

TEMPERATURE (°C)
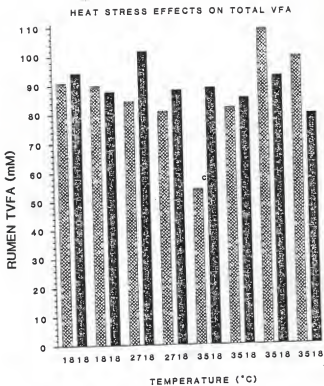
Figure 7. Heat stress effects on rumen acetate.

c. differences between samples at 18C versus 35C (P<.05).

HEAT STRESS EFFECTS ON RUMEN ACETATE

Figure 8. Heat stress effects on rumen propionate.
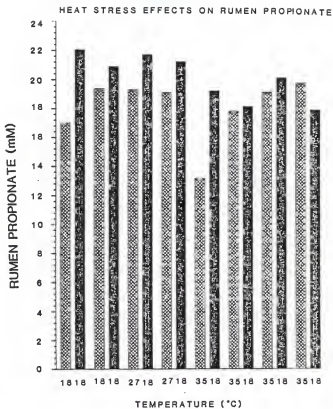
HEAT STRESS EFFECTS ON RUMEN PROPIONATE

RUMEN PROPIONATE (mM)

TEMPERATURE (°C)

Figure 9. Heat stress effects on rumen butyrate.

c. differences between samples at 18C versus 35C (P<.05).

d. differences between HSP versus CP (P<.05).

HEAT STRESS EFFECTS ON RUMEN BUTYRATE

RUMEN BUTYRATE (mM)

TEMPERATURE (°C)

Figure 10. Heat stress effects on portal blood flow.

a. differences between samples at 18C versus 18C (P<.05).

Figure 11. Heat stress effects on arterial glutamate concentration.

a. differences between samples at 18C versus 18C (P<.05).

b. differences between samples at 18C versus 27C (P<.05).

c. differences between samples at 18C versus 35C (P<.05).

d. differences between HSP versus CP (P<.05).

HEAT STRESS EFFECTS ON

ARTERIAL GLUTAMATE CONCENTRATION

ARTERIAL GLUTAMATE (mM)

TEMPERATURE (°C)

Figure 12. Heat stress effects on (A-V) differences of glutamate.

c. differences between 18C versus 35C (P<.05).

HEAT STRESS EFFECTS ON (A-V) DIFFERENCE OF GLUTAMATE
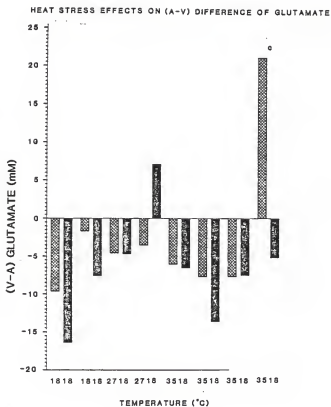
Figure 13. Heat stress effects on net portal
absorption of glutamate.
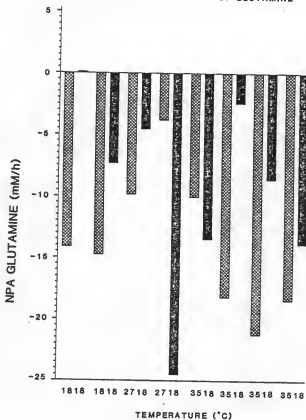
HEAT STRESS EFFECTS ON

NET PORTAL ABSORPTION OF GLUTAMATE

Figure 14. Heat stress effects on arterial glutamine concentrations.
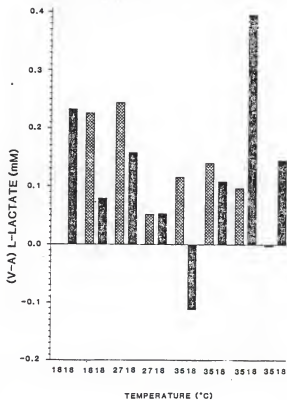
a. differences between samples at 18C versus 18C (P<.05).

Figure 15. Heat stress effects on (A-V) differences of glutamine.

HEAT STRESS EFFECTS ON (A-V) DIFFERENCE OF GLUTAMINE

(V-A) GLUTAMINE (mM)

TEMPERATURE (°C)

Figure 16. Heat stress effects on net portal absorption of glutamine.
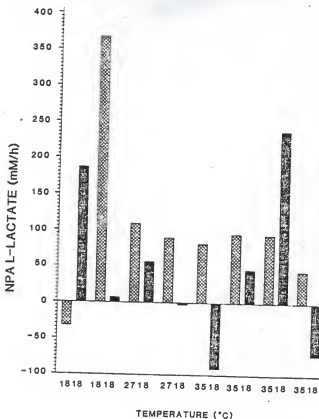
HEAT STRESS EFFECTS ON

NET PORTAL ABSORPTION OF GLUTAMINE

Figure 17. Heat stress effects on arterial L(+) lactate concentration.

HEAT STRESS EFFECTS ON

ARTERIAL L (+) LACTATE CONCENTRATION

Figure 18. Heat stress effects on (A-V) differences
of L(+) lactate.

HEAT STRESS EFFECTS ON

(A–V) DIFFERENCES OF L (+) LACTATE

TEMPERATURE (°C)

Figure 19. Heat stress effects on net portal absorption
of L(+) lactate.

HEAT STRESS EFFECTS ON

NET PORTAL ABSORPTION OF L (+) LACTATE

TABLE 1. Composition of 85% concentrate diet.

Feedstuff	IFN	% of diet dry matter
Corn	4-02-931	75.72
Alfalfa	1-00-071	15.00
Molasses	4-04-696	4.00
Soybean Meal	5-04-612	4.34
Limestone	6-02-632	.58
Dicalcium Phosphate	6-01-080	.06
Trace Mineralized Salt		.30
Vit A,D,E, premix[a]		+

[a] Added to provide 2,200,220 and 22IU/kg of vitamin A,D and E respectively.

HEAT STRESS EFFECTS ON

GUT

NUTRIENT ABSORPTION

INTRODUCTION

The detrimental effects of heat stress on livestock productivity have been well documented (Collier, (1981), Thompson (1973), Roman-Ponce (1977a)). Studies conducted in environmentally-controlled chambers, have identified upper critical temperatures for many production traits. These critical temperatures normally fall between 24 and 27C (Fuquay, (1981)). Roman-Ponce et al. (1978) studied the effects of thermal stress (shade versus no shade conditions) on dairy cows. The results indicate that the average rectal temperatures and respiration rates per minute were lower in animals given access to shade. It was also found that thermal stress reduces fertility and uterine blood flow after injection of estradiol.

Portal blood flow and net absorption of various dietary nutrients have been studied in various species (Huntington (1980), Webster (1973), McGillard (1971)). These reports have quantified absorption of dietary nutrients in natural environments. However, there is limited information available on nutrient metabolism in heat-stressed ruminants. Sands et al. (1982) determined the contribution of blood glucose to heat production and found there is a decreased turnover rate of blood glucose in sheep exposed to 30C with a relative humidity of 70%.

It was the intent of this paper to focus on the influence of environmental temperature on portal blood flow (PBF) and nutrient absorption, and to better understand the relationships between nutrient absorption and heat stress.

MATERIALS AND METHODS

Three Hereford heifers (330kg) were surgically fitted with rumen fistulae, abdominal aorta, hepatic portal, and mesenteric venous catheters (McGillard, 1972). Animals were fed an 85% concentrate and 15% roughage diet with Rumensin added at 44mg/kg (Table 1).

The experiment was divided into a 2d heat stress period (18-35C) and a 2d control period (18C). Intakes during the control period were restricted to what the animals consumed during the heat stress period.

Sampling period

The schedule for sampling and experimental changes are presented in Figure 1. Animals were maintained in an environmentally-controlled chamber. Animals were aacclimated for 48h at 18C and blood samples were taken twice at 0 and 1h (samples (1) and (2)) beginning the heat stress period (HSP). The temperature was increased to 27C and samples at 4 and 5h (samples (3) and (4)) were taken. Finally, the temperature was raised to 35C and samples at 8, 24, 28, and 32h (samples 5-8) were taken. Following sample (8), the temperature was lowered to 18C for a 24h acclimation period. The temperature was maintained at 18C and samples (9-16) were taken, respectively with no increase in temperature constituting the CP. A 48h acclimation at 18C followed the CP. The sampling times were replicated for both the heat stress period and control period.

Sampling procedure

Blood flow was monitered via para-aminohippuric acid. Para-aminohippuric acid (10% w/v) was titrated to pH 7.4 and filtered through a 45 micron filter prior to use. At the beginning of each infusion, a 15ml priming dose was followed by a continuous infusion into the mesenteric venous catheter (Katz and Bergman, 1969) at a rate of .7ml/min for at least 20-30 min prior to sampling.

Respiration rate, heart rate, rectal temperature, and rumen pH were measured 20-30 min before each blood sampling time. Rumen fluid samples were collected using a suction strainer (Raun and Burroughs, 1962) and the pH was taken directly and recorded. A pair of 35ml blood samples were collected simultaneously from the hepatic portal and abdominal aorta catheters into heparinized syringes. Catheters were then refilled with a heparinized saline solution.

Whole blood samples were deproteinized with one-tenth volume 6N $HCLO_4$, centrifuged for 15 min (15,000xg), neutralized with 6N KOH and recentrifuged. The supernatent was poured off and analyzed immediately for glutamine (Lund, 1974) and glutamate (Bernt and Bergmeyer, 1974). The remainder was stored frozen (-20C) and used for analysis of PAH colormetrically (Harvey and Brothers, 1962). Blood (L+) lactate concentrations were determined enzymatically (Bergmeyer, 1974).

The rumen fluid was deproteinized with 25% metaphosphoric acid (Erwin et al., 1961), centrifuged for 15 min at 15,000xg, and stored until VFA analysis by gas chromatography (Hewlett-Packard, 5890A, Palo Alto, Ca.) using 10% SP-1200/1% H_3PO_4 on an 80/100 Chromosorb (2mx6mm glass with a 4mm internal diameter) column (Supelco Inc., Bellefonte, Pa., 16823). Inlet and detector were maintained at 200C; the oven at 140C with a carrier gas (N_2) flow of 40ml/min.

Statistical analysis and calculations

PBF and net absorption from portal-drained viscera were calculated from equations presented by Katz and Bergman, (1975). Data were partitioned into animal, repetition, and sampling time. Comparisons were made within and between sampling periods using appropriate contrasts.

RESULTS AND DISCUSSION

Intakes during the CP were limited to the amount of feed the animal consumed during the HSP. Intakes averaged 4.3+.8 and 3.3+.6 (kg of dry matter+SE) for the HSP and CP, respectively (Table 1).

McDowell et al. (1969) reported a 5% reduction in concentrate consumption and a 22% decline in hay consumption by lactating Holstein cows increased from 18-30C. In contrast, Sanos et al. (1982) reported no decline in intakes when sheep were exposed to 20C and then 30C.

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CONCLUSIONS

In summary, the results conclude:

Rectal temperature, respiration rates, and pulse rates increased during the HSP ($P<.0001$, $P<.0001$, $P<.05$). Rumen pH values were lower for the HSP (5.78-6.10) as compared to the CP (5.88-6.68) ($P<.01$). TVFA were lower only for the first sample taken at 35C (HSP) ($P<.01$). The acetate concentration was lower only for the first sample (5) at 35C ($P<.01$). Concentrations of the propionate were not lower for the HSP ($P<.05$). Concentrations of butyrate were higher for the 35C (HSP) samples than the CP (18) samples ($P<.01$). PBF differences were observed only for 18C sample (2) of the HSP which was higher than the 18C (10) of the CP ($P<.01$). The correlation between PBF and TVFA was not significant (.02). No differences between HSP and CP were observed for the net portal absorption of glutamate, glutamine or $L(+)$ lactate ($P<.05$). The gut uptake of glutamine and glutamate was continuous. Furthermore, $L(+)$ lactate was absorbed rather than utilized.

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Figure 1. Experimental sampling procedure.

Figure 2. Heat stress effects on rectal temperature.

c. differences between samples at 18C versus 35C (P<.05).

d. differences between the HSP and CP (P<.05).
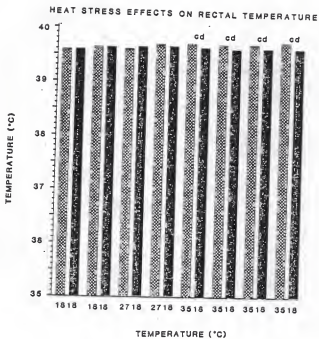
HEAT STRESS EFFECTS ON RECTAL TEMPERATURE
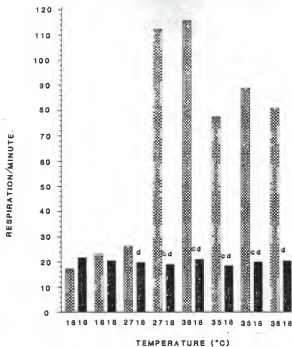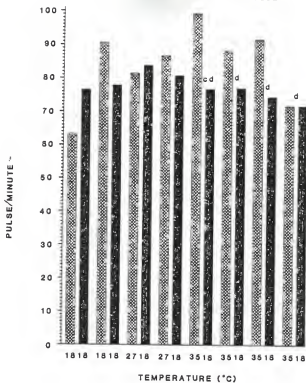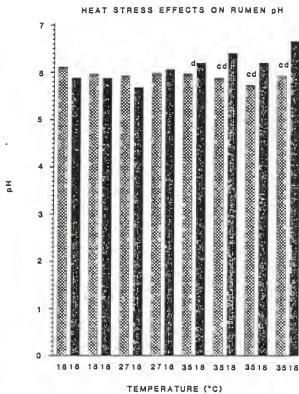
Figure 3. Heat stress effects on respiration.

b. differences between samples at 18C versus 27C (P<.05).

c. differences between samples at 18C versus 35C (P<.05).

d. differences between the HSP and CP (P<.05).

HEAT STRESS EFFECTS ON RESPIRATION

Figure 4. Heat stress effects on pulse.

c. differences between samples at 18C versus 35C (P<.05).

d. differences between the HSP versus CP (P<.05).

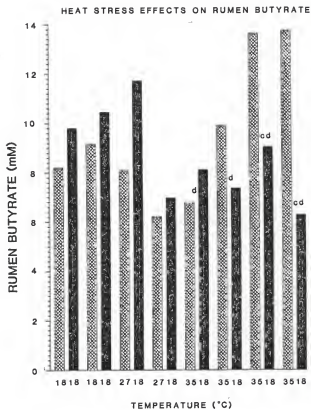Figure 5. Heat stress effects on rumen pH.

c. differences between samples at 18C versus 35C (P<.05).

d. differences between the HSP and CP (P<.05).

HEAT STRESS EFFECTS ON RUMEN pH

pH

TEMPERATURE (°C)

Figure 6. Heat stress effects on total VFA.

c. differences between samples at 18C versus 35C (P<.05).

HEAT STRESS EFFECTS ON TOTAL VFA

Figure 7. Heat stress effects on rumen acetate.

c. differences between samples at 18C versus 35C (P<.05).

HEAT STRESS EFFECTS ON RUMEN ACETATE

RUMEN ACETATE (mM)

TEMPERATURE (°C)

Figure 8. Heat stress effects on rumen propionate.

HEAT STRESS EFFECTS ON RUMEN PROPIONATE

Figure 9. Heat stress effects on rumen butyrate.

c. differences between samples at 18C versus 35C (P<.05).

d. differences between HSP versus CP (P<.05).

HEAT STRESS EFFECTS ON RUMEN BUTYRATE

Figure 10. Heat stress effects on portal blood flow.

a. differences between samples at 18C versus 18C (P<.05).

PORTAL BLOOD FLOW (liter/h)

TEMPERATURE (°C)

Figure 11. Heat stress effects on arterial glutamate concentration.

a. differences between samples at 18C versus 18C (P<.05).

b. differences between samples at 18C versus 27C (P<.05).

c. differences between samples at 18C versus 35C (P<.05).

d. differences between HSP versus CP (P<.05).

HEAT STRESS EFFECTS ON

ARTERIAL GLUTAMATE CONCENTRATION

Figure 12. Heat stress effects on (A-V) differences of
glutamate.

c. differences between 18C versus 35C (P<.05).

HEAT STRESS EFFECTS ON (A-V) DIFFERENCE OF GLUTAMATE

Figure 13. Heat stress effects on net portal
absorption of glutamate.

Figure 14. Heat stress effects on arterial glutamine
concentrations.

a. differences between samples at 18C versus 18C (P<.05).
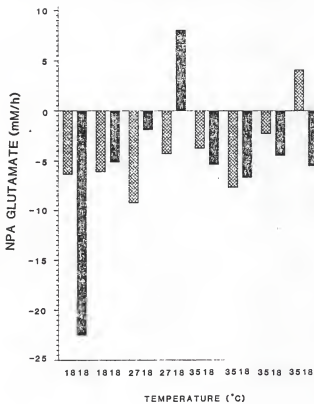
HEAT STRESS EFFECTS ON

NET PORTAL ABSORPTION OF GLUTAMATE

Figure 15. Heat stress effects on (A-V) differences of glutamine.
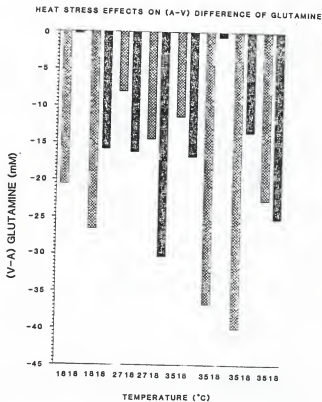
HEAT STRESS EFFECTS ON (A-V) DIFFERENCE OF GLUTAMINE

Figure 16. Heat stress effects on net portal
absorption of glutamine.

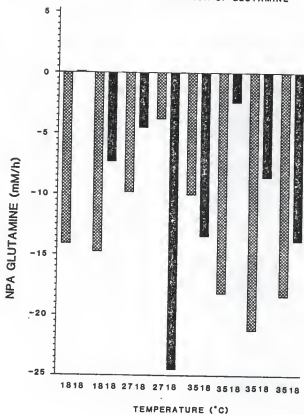HEAT STRESS EFFECTS ON

NET PORTAL ABSORPTION OF GLUTAMINE

Figure 17. Heat stress effects on arterial L(+) lactate
concentration.

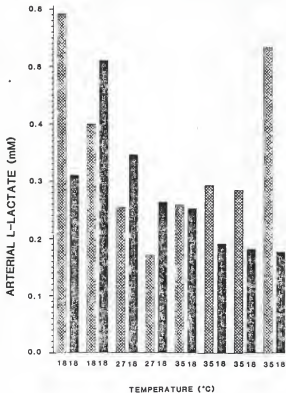HEAT STRESS EFFECTS ON

ARTERIAL L (+) LACTATE CONCENTRATION

Figure 18. Heat stress effects on (A-V) differences
of L(+) lactate.

HEAT STRESS EFFECTS ON
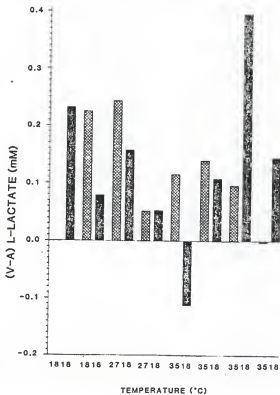
(A-V) DIFFERENCES OF L (+) LACTATE

Figure 19. Heat stress effects on net portal absorption
of L(+) lactate.

HEAT STRESS EFFECTS ON

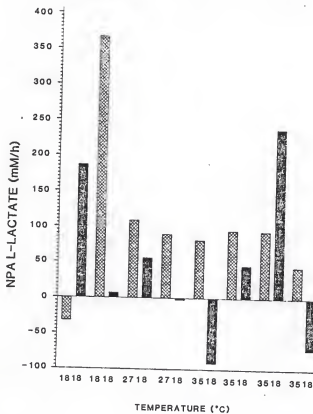NET PORTAL ABSORPTION OF L (+) LACTATE

TABLE 1. Composition of 85% concentrate diet.

Feedstuff	IFN	% of diet dry matter
Corn	4-02-931	75.72
Alfalfa	1-00-071	15.00
Molasses	4-04-696	4.00
Soybean Meal	5-04-612	4.34
Limestone	6-02-632	.58
Dicalcium Phosphate	6-01-080	.06
Trace Mineralized Salt		.30
Vit A,D,E, premix[a]		+

[a] Added to provide 2,200,220 and 22IU/kg of vitamin A,D and E respectively.

THE ROLE OF SOLUBLE CARBOHYDRATES
IN LACTIC ACID PRODUCTION

by

ANDRA JANE CULLEN

B.S., California State University
at Fresno, 1983

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment

of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Science

Kansas State University
Manhattan, Kansas

1985

ABSTRACT

The purpose of this study was to determine the lactic acid (D and L)
production from various grain and by-products in vitro, to better understand
the relationship between soluble carbohydrates and lactic acid formation.
In vitro incubations revealed that hexose sugars exhibited a greater lactic
acid production at 12h than pentose sugars. The pentose sugars were not
readily fermented to lactic acid. In vitro incubations of grains and by-
products revealed a ranking of total lactic acid production at 12h: steam-
flaked barley = whole barley = wheat > citrus pulp > beet pulp = corn >
high-moisture corn > milo. A segment of this experiment involved the
extraction of the soluble fraction via hot (70C) water. The extracted
water-soluble fraction and the insoluble fraction were lyphilized and
used for in vitro incubations as well as carbohydrate analysis via HPLC.
Individual in vitro analysis of the soluble fraction and insoluble fraction
revealed that lactic acid concentrations of the insoluble fractions were
lower than the soluble fractions (P<.05). Analysis revealed that soluble
sugars accounted for only about one-third of the soluble fraction. In vitro
incubations were performed on combinations of the least lactic acid provoca-
tive insoluble grains (milo, corn, high-moisture corn) with the most lactic
acid provocative soluble fractions (steam-flaked barley, whole barley,
wheat). The greatest response was seen with the corn insoluble extract. In
general, the milo extract and high-moisture corn showed little if any re-
sponses to the soluble fraction additions at 12h.

Results indicate that certain grains contain a higher percent of water
soluble materials than others. In order to predict lactic acid production,
it is important to know the percent water soluble material of the whole
grain as well as the fermentable carbohydrates which composite the soluble

fractions. Water soluble materials are important for the initiation of the lactic acid production; thereafter, the production is dependent on the fermentability of the grain starch.